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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/18</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/12628</b> <b>(43) International Publication Date:</b> 10 April 1997 (10.04.97)
<b>(21) International Application Number:</b> PCT/US96/15899 <b>(22) International Filing Date:</b> 4 October 1996 (04.10.96) <b>(30) Priority Data:</b> 60/004,820 5 October 1995 (05.10.95) US <b>(71) Applicants:</b> GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US). THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02115 (US). <b>(72) Inventors:</b> BUNTING, Stuart; 1106 Date Street, Montara, CA 94037 (US). GOLD, Herman, K.; 22 Hyslop Road, Brookline, MA 02146 (US). LEINBACH, Robert, C.; 67 Frances Street, Brookline, MA 02146 (US). SCHWALL, Ralph, H.; 400 Griffin Avenue, Pacifica, CA 94044 (US). <b>(74) Agents:</b> MARSCHANG, Diane, L. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> METHODS AND COMPOSITIONS FOR TREATING VASCULAR STENOSIS  <b>(57) Abstract</b>  Methods for treating vascular stenosis using hepatocyte growth factor ("HGF") are provided. In the methods, HGF can be administered to enhance re-surfacing of blood vessels damaged or traumatized, for instance, by vascular surgery or angioplasty. Articles of manufacture and kits containing HGF are also provided.		

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## METHODS AND COMPOSITIONS FOR TREATING VASCULAR STENOSIS

Field of the Invention

The invention relates generally to methods and compositions which can be employed to enhance re-surfacing of blood vessels and treat blood vessel stenosis.

Background of the Invention

Hepatocyte growth factor ("HGF") functions as a growth factor for particular tissues and cell types. HGF was identified initially as a mitogen for hepatocytes [Michalopoulos et al., Cancer Res., 44:4414-4419 (1984); Russel et al., J. Cell. Physiol., 119:183-192 (1984); Nakamura et al., Biochem. Biophys. Res. Comm., 122:1450-1459 (1984)]. Nakamura et al., *supra*, reported the purification of HGF from the serum of partially hepatectomized rats. Subsequently, HGF was purified from rat platelets, and its subunit structure was determined [Nakamura et al., Proc. Natl. Acad. Sci. USA, 83:6489-6493 (1986); Nakamura et al., FEBS Letters, 224:311-316 (1987)]. The purification of human HGF ("huHGF") from human plasma was first described by Gohda et al., J. Clin. Invest., 81:414-419 (1988).

Both rat HGF and huHGF have been molecularly cloned, including the cloning and sequencing of a naturally occurring variant lacking 5 amino acids designated "delta5 HGF" [Miyazawa et al., Biochem. Biophys. Res. Comm., 163:967-973 (1989); Nakamura et al., Nature, 342:440-443 (1989); Seki et al., Biochem. Biophys. Res. Commun., 172:321-327 (1990); Tashiro et al., Proc. Natl. Acad. Sci. USA, 87:3200-3204 (1990); Okajima et al., Eur. J. Biochem., 193:375-381 (1990)].

The mature form of huHGF, corresponding to the major form purified from human serum, is a disulfide linked heterodimer derived by proteolytic cleavage of the human pro-hormone between amino acids R494 and V495. This cleavage process generates a molecule composed of an  $\alpha$ -subunit of 440 amino acids ( $M_r$  69 kDa) and a  $\beta$ -subunit of 234 amino acids ( $M_r$  34 kDa). The nucleotide sequence of the huHGF cDNA reveals that both the  $\alpha$ - and the  $\beta$ -chains are contained in a single open reading frame coding for a pre-pro precursor protein. In the predicted primary structure of mature huHGF, an interchain S-S bridge is formed between Cys 487 of the  $\alpha$ -chain and Cys 604 in the  $\beta$ -chain [see Nakamura et al., Nature, *supra*]. The N-terminus of the  $\alpha$ -chain is preceded by 54 amino acids, starting with a methionine group. This segment includes a characteristic hydrophobic leader (signal) sequence of 31 residues and the prosequence. The  $\alpha$ -chain starts at amino acid (aa) 55, and contains four kringle domains. The kringle 1 domain extends from about aa 128 to about aa 206, the kringle 2 domain is between about aa 211 and about aa 288, the kringle 3 domain is defined as extending from about aa 303 to about aa 383, and the kringle 4 domain extends from about aa 391 to about aa 464 of the  $\alpha$ -chain.

The definition of the various kringle domains is based on their homology with kringle-like domains of other proteins (such as prothrombin and plasminogen), therefore, the above limits are only approximate. To date, the function of these kringles has not been determined. The  $\beta$ -chain of huHGF shows high homology to the catalytic domain of serine proteases (38% homology to the plasminogen serine protease domain). However, two of the three residues which form the catalytic triad of serine proteases are not conserved in huHGF. Therefore, despite its serine protease-like domain, huHGF appears to have no proteolytic

activity, and the precise role of the  $\beta$ -chain remains unknown. HGF contains four putative glycosylation sites, which are located at positions 294 and 402 of the  $\alpha$ -chain and at positions 566 and 653 of the  $\beta$ -chain.

In a portion of cDNA isolated from human leukocytes, in-frame deletion of 15 base pairs was observed. Transient expression of the cDNA sequence in COS-1 cells revealed that the encoded HGF molecule (delta5 HGF) lacking 5 amino acids in the kringle 1 domain was fully functional [Seki et al., *supra*].

A naturally occurring huHGF variant has been identified which corresponds to an alternative spliced form of the huHGF transcript containing the coding sequences for the N-terminal finger and first two kringle domains of mature huHGF [Chan et al., *Science*, 254:1382-1385 (1991); Miyazawa et al., *Eur. J. Biochem.*, 197:15-22 (1991)]. This variant, designated HGF/NK2, has been proposed to be a competitive antagonist of mature huHGF.

Comparisons of the amino acid sequence of rat HGF with that of huHGF have revealed that the two sequences are highly conserved and have the same characteristic structural features. The length of the four kringle domains in rat HGF is exactly the same as in huHGF. Furthermore, the cysteine residues are located in exactly the same positions, an indication of similar three-dimensional structures [Okajima et al., *supra*; Tashiro et al., *supra*].

HGF and HGF variants are described further in U.S. Patent Nos. 5,227,158, 5,316,921, and 5,328,837.

The HGF receptor has been identified as the product of the c-Met proto-oncogene [Bottaro et al., *Science*, 251:802-804 (1991); Naldini et al., *Oncogene*, 6:501-504 (1991); WO 92/13097 published August 6, 1992; WO 93/15754 published August 19, 1993]. The receptor is usually referred to as "c-Met" or "p190<sup>MET</sup>" and typically comprises, in its native form, a 190-kDa heterodimeric (a disulfide-linked 50-kDa  $\alpha$ -chain and a 145-kDa  $\beta$ -chain) membrane-spanning tyrosine kinase protein [Park et al., *Proc. Natl. Acad. Sci. USA*, 84:6379-6383 (1987)]. Several truncated forms of the c-Met receptor have also been described [WO 92/20792; Prat et al., *Mol. Cell. Biol.*, 11:5954-5962 (1991)].

The binding activity of HGF to its receptor is believed to be conveyed by a functional domain located in the N-terminal portion of the HGF molecule, including the first two kringles [Matsumoto et al., *Biochem. Biophys. Res. Commun.*, 181:691-699 (1991); Hartmann et al., *Proc. Natl. Acad. Sci.*, 89:11574-11578 (1992); Lokker et al., *EMBO J.*, 11:2503-2510 (1992); Lokker and Godowski, *J. Biol. Chem.*, 268:17145-17150 (1991)]. The c-Met protein becomes phosphorylated on tyrosine residues of the 145-kDa  $\beta$ -subunit upon HGF binding.

Various biological activities have been described for HGF and its receptor (see, generally, Chan et al., *Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the C-Met Receptor*, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 67-79]. It has been observed that levels of HGF increase in the plasma of patients with hepatic failure [Gohda et al., *supra*] and in the plasma [Lindroos et al., *Hepatology*, 13:734-750 (1991)] or serum [Asami et al., *J. Biochem.*, 109:8-13 (1991)] of animals with experimentally induced liver damage. The kinetics of this response are usually rapid, and precedes the first round of DNA synthesis during liver regeneration. HGF has also been shown to be a mitogen for certain cell types, including melanocytes, renal tubular cells, keratinocytes, certain endothelial cells and cells of epithelial origin [Matsumoto et al., *Biochem. Biophys. Res. Commun.*, 176:45-51 (1991); Igawa et al., *Biochem. Biophys. Res. Commun.*, 174:831-838 (1991); Han et al., *Biochem.*, 30:9768-9780 (1991); Rubin et al., *Proc. Natl. Acad.*

Sci. USA, 88:415-419 (1991)]. Both HGF and the c-Met protooncogene have been postulated to play a role in microglial reactions to CNS injuries [DiRenzo et al., Oncogene, 8:219-222 (1993)].

HGF can also act as a "scatter factor", an activity that promotes the dissociation of epithelial and vascular endothelial cells *in vitro* [Stoker et al., Nature, 327:239-242 (1987); Weidner et al., J. Cell Biol., 111:2097-2108 (1990); Naldini et al., EMBO J., 10:2867-2878 (1991); Giordano et al., Proc. Natl. Acad. Sci. USA, 90:649-653 (1993)]. Moreover, HGF has recently been described as an epithelial morphogen [Montesano et al., Cell, 67:901-908 (1991)]. Therefore, HGF has been postulated to be important in tumor invasion [Comoglio, Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the C-Met Receptor, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 131-165].

Vascular stenosis (and restenosis) is a pathological condition which often results from vascular trauma or damage to blood vessel walls. Vascular trauma or damage is relatively common when a patient undergoes vascular surgery or other therapeutic techniques such as angioplasty.

#### Summary of the Invention

The invention provides methods for treating vascular stenosis comprising administering to a mammal an effective amount of HGF. The HGF alone may be administered to the mammal, or alternatively, may be administered to the mammal in combination with other therapies and/or pharmacologic agents.

The invention also provides articles of manufacture and kits which contain HGF.

Although not being bound by any particular theory, it is presently believed that the HGF can be used to enhance re-surfacing of damaged blood vessels. By using the HGF to re-surface the damaged vessel relatively rapidly, it is believed that surrounding muscle cells will be unable to proliferate or form a stenosis at the site of the damage.

#### Detailed Description of the Invention

##### I. Definitions

As used herein, the terms "hepatocyte growth factor" and "HGF" refer to a growth factor typically having a structure with six domains (finger, Kringle 1, Kringle 2, Kringle 3, Kringle 4 and serine protease domains). Fragments of HGF constitute HGF with fewer domains and variants of HGF may have some of the domains of HGF repeated; both are included if they still retain their respective ability to bind a HGF receptor. The terms "hepatocyte growth factor" and "HGF" include hepatocyte growth factor from humans ("huHGF") and any non-human mammalian species, and in particular rat HGF. The terms as used herein include mature, pre, pre-pro, and pro forms, purified from a natural source, chemically synthesized or recombinantly produced. Human HGF is encoded by the cDNA sequence published by Miyazawa et al., 1989, *supra*, or Nakamura et al., 1989, *supra*. The sequences reported by Miyazawa et al. and Nakamura et al. differ in 14 amino acids. The reason for the differences is not entirely clear; polymorphism or cloning artifacts are among the possibilities. Both sequences are specifically encompassed by the foregoing terms. It will be understood that natural allelic variations exist and can occur among individuals, as demonstrated by one or more amino acid differences in the amino acid sequence of each individual. The terms "hepatocyte growth factor" and "HGF" specifically include the delta5 huHGF as disclosed by Seki et al., *supra*.

The terms "HGF receptor" and "c-Met" when used herein refer to a cellular receptor for HGF, which typically includes an extracellular domain, a transmembrane domain and an intracellular domain, as well as variants and fragments thereof which retain the ability to bind HGF. The terms "HGF receptor" and "c-Met" include the polypeptide molecule that comprises the full-length, native amino acid sequence encoded by the gene variously known as p190<sup>MET</sup>. The present definition specifically encompasses soluble forms of HGF receptor, and HGF receptor from natural sources, synthetically produced *in vitro* or obtained by genetic manipulation including methods of recombinant DNA technology. The HGF receptor variants or fragments preferably share at least about 65% sequence homology, and more preferably at least about 75% sequence homology with any domain of the human c-Met amino acid sequence published in Rodrigues et al., *Mol. Cell. Biol.* 11:2962-2970 (1991); Park et al., *Proc. Natl. Acad. Sci.* 84:6379-6383 (1987); or Ponzetto et al., *Oncogene* 6:553-559 (1991).

The term "vascular stenosis" is used in a broad sense and refers to a pathological process in which the cavity of a blood vessel is narrowed and which usually results in a pathological condition characterized by impaired flow through the vessel.

The terms "treating," "treatment," and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy.

The term "mammal" as used herein refers to any mammal classified as a mammal, including humans, cows, horses, dogs and cats. In a preferred embodiment of the invention, the mammal is a human.

## II. Compositions and Methods of the Invention

The present invention provides methods for treating vascular stenosis using hepatocyte growth factor, referred to hereinafter as "HGF". The HGF useful in the practice of the present invention can be prepared in a number of ways. For instance, the HGF can be prepared using an isolated or purified form of HGF. Methods of isolating and purifying HGF from natural sources are known in the art. Such isolation and purification methods can be employed for obtaining HGF from serum or plasma. Alternatively, HGF can be chemically synthesized and prepared using recombinant DNA techniques known in the art and described in further detail in the Example below.

The HGF may be from human or any non-human species. For instance, a mammal may have administered HGF from a different mammalian species (e.g., rats can be treated with human HGF). Preferably, however, the mammal is treated with homologous HGF (e.g., humans are treated with human HGF) to avoid potential immune reactions to the HGF. In the methods, HGF is typically administered to a mammal having vascular damage or trauma, or is believed to be at risk of developing vascular damage or trauma. It is contemplated that the methods of the invention will be particularly useful in combination with therapeutic techniques such as angioplasty.

The HGF is preferably administered to the mammal in a pharmaceutically-acceptable carrier. Suitable carriers and their formulations are described in *Remington's Pharmaceutical Sciences*, 16th ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include liquids such as saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. The formulation may also

comprise a lyophilized powder. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of HGF being administered.

The HGF can be administered to the mammal by injection (e.g. intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular), or by other methods such as infusion that ensure its delivery to the bloodstream in an effective form. Optionally, the HGF may be administered by multiple routes such as both intraarterially and intravenously.

Effective dosages and schedules for administering the HGF may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of HGF that must be administered will vary depending on, for example, the mammal which will receive the HGF, the route of administration, the particular type of HGF used and other drugs being administered to the mammal. A typical daily dosage of the HGF used alone might range from about 1  $\mu$ g/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

HGF may also be administered along with other pharmacologic agents used to treat vascular conditions. The HGF may be administered sequentially or concurrently with one or more other pharmacologic agents. The amounts of HGF and pharmacologic agent depend, for example, on what type of drugs are used, the disease being treated, and the scheduling and routes of administration.

Following administration of HGF to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner.

In another embodiment of the invention, there are provided articles of manufacture and kits containing materials useful for treating vascular stenosis. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating vascular stenosis. The active agent in the composition is HGF. The label on the container indicates that the composition is used for treating vascular stenosis, and may also indicate directions for *in vivo* use, such as those described above.

The kit of the invention comprises the container described above and a second container comprising a buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All reference citations herein are incorporated by reference.

#### Example

Recombinant human HGF ("rhHGF") was produced in CHO cells using a procedure modified from Naka et al., *J. Biol. Chem.*, 267:20114-20119 (1992). rhHGF-transfected cells were grown in a 400 L bioreactor in medium containing 2% fetal bovine serum for 8 days. Culture supernatant containing rhHGF was concentrated and clarified, then conditioned by the addition of solid NaCl to 0.3 M. rhHGF was

then purified in a single step using cation exchange chromatography. Conditioned, concentrated culture supernatant was loaded onto a column of S-Sepharose Fast Flow equilibrated in 20 mM Tris, pH 7.5, 0.3 M NaCl. After washing out unbound protein, rhuHGF was eluted in a linear gradient from 20 mM Tris, pH 7.5, 0.3 M NaCl to 20 mM Tris, pH 7.5, 1.2 M NaCl. rhuHGF-containing fractions were pooled based on SDS-PAGE analysis. The S Sepharose Fast Flow pool was concentrated and exchanged into 20 mM Tris, pH 7.5, 0.5 M NaCl by gel filtration on Sephadex G25 to a final concentration of about 3-5 mg/ml. A rhuHGF stock solution was then prepared by diluting the rhuHGF in buffer (0.5% bovine serum albumin, 0.05% Tween-20, 0.01% Thimersol in PBS).

To test the efficacy of rhuHGF to accelerate endothelial cell re-surfacing of denuded arterial surfaces *in vivo*, anesthetized rats were subjected to arterial balloon injury. A 2mm x 2mm angioplasty balloon was inserted antegrade into the abdominal aorta of the animals through a renal artery after ligation of distal side branches. Three inflations were made at 2 atm/30 seconds followed by pullback at 0.5 atm. The denuded segment was then isolated between snares and 0.2 ml of rhuHGF solution (1 mg/ml) was instilled into the artery ("IA") and incubated for 30 minutes. In six additional animals, rhuHGF was further administered at 0.5 mg/day intravenously ("IV") for 5 days. Control animals received vehicle instillation (0.5M saline, 20mM Tris) only.

Reanesthetized animals were injected at 1 week with Evans Blue, and the harvested aortas were analyzed morphometrically for endothelial cell regrowth expressed as % coverage and length of the denuded area. The results are shown below in Table 1.

TABLE 1

	Number of Animals	% Surface Covered	Length of Denuded Segment (cm)
Control	9	30 +/- 15	1.7 +/- 0.26
rhuHGF(IA)	12	46 +/- 17*	1.5 +/- 0.2
rhuHGF (IA & IV)	6	67 +/- 18**	1.3 +/- 0.07

\*p<0.04  
vs. control

p=0.03 vs. control

\*\*p<0.001 vs. control

The data showed that the HGF enhanced aortic resurfacing by Day 7. Resurfacing was markedly enhanced when multiple doses of the HGF were administered intravenously following the initial intraarterial administration of HGF.



What is claimed is:

1. A method of treating vascular stenosis in a mammal, comprising administering an effective amount of hepatocyte growth factor ("HGF") to the mammal.
2. The method of claim 1 wherein said HGF is human HGF.
- 5 3. The method of claim 1 wherein said HGF is administered intraarterially.
4. The method of claim 3 wherein said HGF is further administered intravenously.
5. An article of manufacture, comprising:
  - a container;
  - a label on said container; and
  - 10 a composition contained within said container;wherein the composition is effective for treating vascular stenosis, the label on said container indicates that the composition can be used for treating vascular stenosis, and the active agent in said composition comprises HGF.
6. The article of manufacture of claim 5 further comprising instructions for administering the HGF  
15 to a mammal.
7. A kit, comprising:
  - a first container, a label on said container, and
  - a composition contained within said container;
  - wherein the composition is effective for treating vascular stenosis, the label on said container  
20 indicates that the composition can be used for treating vascular stenosis, and the active agent in said composition comprises HGF;
  - a second container comprising a pharmaceutically-acceptable buffer; and
  - instructions for using the HGF to treat vascular stenosis.

# INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/15899

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 90, no. 5, 1 March 1990, WASHINGTON US, pages 1937-1941, XP002022436 GRANT D.S.: "Scatter factor induces blood vessel formation in vivo" see the whole document	1-7
P,X	68TH SCIENTIFIC SESSION OF THE AMERICAN HEART ASSOCIATION, ANAHEIM, CALIFORNIA, USA, NOVEMBER 13-16, 1995. CIRCULATION 92 (8 SUPPL.). 1995. 1750, XP002022818 GARABEDIAN H D ET AL: "Recombinant hepatocyte growth factor accelerates endothelial cell regrowth in vivo following arterial injury." see the whole document	1-7

-/-

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

\* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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Date of the actual completion of the international search

16 January 1997

Date of mailing of the international search report

27. 01. 97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+ 31-70) 340-3016

Authorized officer

Moreau, J

# INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/15899

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHEMICAL ABSTRACTS, vol. 125, no. 23, 2 December 1996 Columbus, Ohio, US; abstract no. 294033, ISHIDA, MARI ET AL: "Hepatocyte growth factor for prevention and treatment of thrombosis and vascular endothelial cell damage -related diseases" XP002022819 see abstract & JP 08 231 416 A (MITSUBISHI CHEM CORP, JAPAN)	1-7
P,X	--- CHEMICAL ABSTRACTS, vol. 124, no. 15, 9 April 1996 Columbus, Ohio, US; abstract no. 192980, KIDA, IWA0 ET AL: "Restenosis after percutaneous transluminal coronary angioplasty. Trial of gene therapy" XP002022820 see abstract & KEKKAN TO NAIHI (1996), 6(1), 47-55, 1996, -----	1-7

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/15899

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-4  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1-4 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.